Original Article

Analytical Evaluation of *Mycobacterium tuberculosis* Detection in a Local Comprehensive Tuberculosis Center Following the Introduction of GeneXpert: A Cartridge-Based Nucleic Acid Amplification Test

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Abstract

Background: The policies and methods for preliminary evaluation of *Mycobacterium tuberculosis* (MTB) are evolving as TB becomes resistant to drugs worldwide. Evaluating the effectiveness of New GeneXpert as a first-line protocol for replacing AFB/ZN microscopy in TB evaluation for more system future rollout was paramount for effective TB detection. **Aim:** The research was a retrospective analytical cohort study, evaluating the detection rates of MTB using Direct-AFB microscopy and GeneXpert among samples from TB suspects. **Materials and Methods:** Data were collated from the National TB and Leprosy Control Programme register and laboratory records. The study period was from February 2015 to October 2017. The study period was divided into three, 11 months each, representing different preliminary testing policy. Proportions and rates were determined using Microsoft Excel and Chi-square analysis. A $P \leq 0.05$ was considered statistically significant. **Results:** A total of 1931 sample results were analyzed, of which 99.9% were sputum. Nearly 502,578 and 177 samples underwent AFB/ZN microscopy technique, whereas 0, 40, and 634 samples underwent GeneXpert for the respective three cohort periods. The results showed that MTB was present at rates of 8.17%, 5%, and 3.39% for ZN, whereas GeneXpert was 15% and 12.6% for the 2nd and 3rd cohort periods only. In addition, 10% detected by GeneXpert were rifampicin drug resistant, and 50% (4) were placed on therapy for resistant strain GeneXpert improved TB detection significantly. **Conclusion:** GeneXpert could improve the detection of MTB/RIF strains in developing countries through partnership and global funding for TB/ART centers.

Keywords: GeneXpert, rifampicin resistance, tuberculosis

Introduction

Nigeria is among the first four countries with the highest burden of tuberculosis (TB) in the world. A significant number of Nigerians with HIV may develop TB disease, while about 39,000 cases die from the disease each year. Nigeria has been noted to be among the top ten countries accounting for 77% of the global gap in TB case detection and notification in 2016, leading to 8% of the 4.3 million missed TB cases globally.[1]

In 1993, National TB and Leprosy Control Programme (NTBLCP) initiated the directly observed therapy (DOT) strategy which to improve the management of TB, leading to the reduction of new cases by to 20% in 2006.[2] The HIV burden in Nigeria seems to also prevent the drastic reduction of TB disease in Nigeria. Nigeria's HIV seroprevalence rate was reported to be as low as 4.4% in 2006, although, Benue state in Nigeria, had a 10% sero-prevalence rate. Nigeria ranks third among countries in the world today, as regard to the burden of HIV in the world. It is estimated that there are 3 million infected persons.[2-4] In Nigeria, about 21% of all TB patients are HIV infected.[2-4]

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TB resistance to drugs was reported in Nigeria, 30 years ago.[5] Even in recent times, there is the belief that the resistance rate of TB may have increased. The developed and highly civilized society is not left out in the battle of MDR-TB as human migration continues to increase.

The WHO projected that a fifth part, out of the estimated 480,000 new cases of multi-drug resistant (MDR-TB), may be rifampicin specific by 2005. Three countries, India, China, and the Russian Federation, accounted for almost half (45%) of the total burden.[6] GeneXpert, otherwise known as cartridge-based nucleic acid amplification test, is a diagnostic tool used currently to test for TB bacilli and its phenotypic drug susceptibility and resistance even for microscopy smear-negative cases. This principle of gene amplification testing was also employed for SARs-CoV-2 testing in the recent pandemic.[7] Due to the delays emanating from drug resistance testing by culture techniques, a study reported that only 20% of MDR-TB cases were enrolled in treatment programs.[6] Other studies have reported success rates <20% in treatment with fluoroquinolones for MDR-TB.[8,9] These are unfortunate results due to the low detection rate in the absence of gene amplification system; therefore, early detection for MDR-TB is paramount in reducing the prevalence and incidences of resistant TB among the population at risk, which necessitated the development of GeneXpert.

Besides longer treatment duration, cost implications, and adverse effects of drugs, the main difficulty in managing MDR- and extensively drug-resistant-TB is to identify at least four active drugs necessary for an effective regimen.[8-13]

Global Fund has been able to support the roll-out of 185 GeneXpert machines within Nigeria in 2016 to improve TB screening services at all ART centers. As a part of the new strategy, NACA's GeneXpert technical team, through its mandate, was able to select the sites for GeneXpert installation. General Hospital, Biu, was among the first four sites chosen in Borno State. Others were to be rolled out by National TB, Leprosy, and Buruli Ulcer Control Program NTBLCP on later dates. Based on this background, we hereby use the opportunity to carry out a retrospective evaluation of the effectiveness of changing the *Mycobacterium* TB (MTB) detection policy in a local TB comprehensive centre, for which many facilities in remote settings in many developing countries could use as a model for improving TB testing services.

The main objective is to determine any changes in the TB detection rate at the three phases of the TB local operational testing policy.

Materials and Methods

The study was carried out in General Hospital Biu, which is located in northeast Nigeria. The region has been ravaged by militant insurgency and serves the region as a stable center for TB service. It has a DOT centre that takes care of patients from most areas in Southern Borno State. It consists of four trained staff in TB comprehensive care centre, attached to a specialized, well-equipped laboratory for TB bacilli diagnosis, and a comprehensive HIV care centre. The TB center was recently upgraded in 2017 for the detection of TB drug-resistant strains using GeneXpert with the support of an international organization in partnership with NTBLC of the Federal Ministry of Health.

The GeneXpert system integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences using real-time polymerase chain reaction (PCR) and reverse transcriptase PCR. The system requires the use of single-use disposable GeneXpert MTB/Rif cartridges that hold the PCR reagents and host the PCR process.

The study was a retrospective cohort study on the detection rates of TB in patient samples between GeneXpert and Ziehl-Nelson Direct acid-fast bacilli staining technique. Records were collected from the NTBLCP register and from the laboratory record on TB testing. Testing was done freely without cost for the patients as a policy.

The study period spanned from February 2015 to October 2017, a total of 33 months. Each cohort studied period was composed of 11 months each.

The first period was from February 2015 to December 2016, which was termed AFB/NO GeneXpert period. The testing operational guideline at the local center was direct microscopy using the acid-fast technique only.

The second period spanned from January 2016 to November 2016, which was termed AFB/GeneXpert referral period. The testing operational guideline was both direct microscopy using the microscopy with acid-fast technique and GeneXpert only on referral to the National TB/Leprosy control center.

The third cohort period of study was from December 2016 to October 2017, which was termed local centre GeneXpert. This period was characterized by all samples subjected to GeneXpert only as a guideline for testing in that period.

The patients were those who met clinical criteria for testing for TB, which include chronic productive cough with or without hemoptysis, or those that do not respond to regular antibiotics, associated chronic weight loss, and night sweat, and patients who had HIV with or without medications had a chronic cough were included as well as those who had chest X-ray features suggestive of TB infection.

At General Hospital Biu, the acid-fast bacilli microscopy was performed by an experienced chief laboratory scientist following the guidelines set by the national testing protocol, while the sputum sample for the Xpert was processed according to the manufacturer's guidelines.

Statistical analysis was performed using Microsoft Excel and Chi-square analysis. A $P \leq 0.05$ was considered statistically significant.

Results

A total of 1931 samples of suspected patients were tested for MTB between February 2015 and October 2017. These samples were from three cohort periods.

Table 1 shows the total number of patients that were screened for TB following the National TB guideline. A total of 76 and 82 samples were positive for TB bacilli out of 1257 and 674 samples when subjected to AFB-microscopy and GeneXpert, respectively. Table 2 results showed a significant improvement in the detection rate when GeneXpert became installed in the local TB comprehensive center, as shown in cohort 3. Chi-square analysis showed an increased detection rate from 8.17% in cohort 1 to 12%–15% in cohorts 2 and 3,which was statistically significant. $P = 0.000$ which was <0.005.

The results in Table 3 further highlight the additional advantage of using GeneXpert for detecting TB, where almost 10% of the patient's sample was positive for rifampicin-multidrug-resistant bacilli. The period of AFB-microscopy technique could not offer MDR-TB testing on site; this potentiates a high treatment failure rate and also further complications of the disease.

Discussion

The result of our study revealed that the use of GeneXpert as a new testing policy in general hospitals could substantially increase the detection for confirmed TB cases. According to this study from Biu General Hospital, the use of GeneXpert had resulted in improvement in the detection of TB cases among samples received from the suspected populace, compared to the acid-fast bacilli microscopy technique. Statistical analysis further affirms that the change in policy using Genexpert as first line for the evaluation of TB among samples improves TB detection. Another study also demonstrated a higher rate of 28% among patient samples using GeneXpert to detect TB.[14] The improvement was notably due to its very high specificity, which is said to be 99%, as revealed by some meta-analytical studies.[15-17] This study showed that pilot models as this could increase the detection of TB among the populace at risk, especially communities with a high burden of TB diseases. The high rate of turnover for testing samples could assist centres to subject more samples for testing within a day, as seen in our study. The study also revealed that secondary health facility upgrades could assist in reducing workload from tertiary centers that have GeneXpert or serve as referral systems for the secondary centre, with reference to our experience. It would bring drug resistance testing closer to the people at risk, consequently preventing community transmission during referrals and improving the registry of those treated with MDR-TB.

In countries with a high prevalence of TB such as Nigeria, most suspected TB cases were assessed by sputum smear microscopy and chest X-ray; thereafter, the patients are often placed on TB treatment based on persistent cough or abnormal chest X-ray

Table 1: Number of sample testing among Cohort periods

List of Cohorts	Testing operational Guideline	Total tested samples	AFB tested samples	Positive results Scanty-3+	GeneXpert tested	GeneXpert positive
Cohort 1	AFB only	502	502	41	0	0
Cohort 2	AFB Gene-Xpert	618	578	29	40	6
Cohort 3	GeneXpert	811	177	6	634	76
Total		1931	1257	76	674	82

The zero; indicated that GeneXpert was not available and therefore was not a Guideline protocol

Table 2: The proportion of positive results with GeneXpert and acid-fast bacilli-microscopy

List of Cohorts	Testing operational Guideline	Proportions tested positive with AFB (%)	The proportion tested positive with GeneXpert (%)
Cohort 1	AFB only	8.17	0
Cohort 2	AFB Gene-Xpert	5	15
Cohort 3	GeneXpert	3.39	12.6

The zero; indicated that GeneXpert was not available and therefore was not a Guideline protocol. At P=0.000229 there was significant improvement in the detection rate by GeneXpert; at P=0.000244 showed that policy changes had improved TB detection from 1st Cohort to the 3rd Cohort group. TB: Tuberculosis, AFB: Acid-fast bacilli

Table 3: Proportion of multidrug-resistant mycobacterium bacilli and treatment group

Testing operational Guideline	Total tested positive with GeneXpert	The proportion (%)
GeneXpert	82	12.16
No of Rifampicin-MDRT	8	10
On medication against MDRT with culture monitoring	4	5

TB: Tuberculosis, MDRT: Multidrug-resistant TB

alone with or without AFB microscopy until the recent era of GeneXpert. The current recommendation for the treatment of newly diagnosed TB patients includes combination therapy using rifampicin, isoniazid, pyrazinamide, and ethambutol or streptomycin for two months, followed by isoniazid and rifampicin for four months or isoniazid and ethambutol for six months to reduce rifampicin and other drugs interaction, while for suspected disseminated TB or extrapulmonary TB, treatment is usually longer, even up to 9–12 months.

The propensity for GeneXpert to aptly confirm TB in smear-negative cases proffers the chances of improving early TB case detection, as shown in Table 2. However, due to financial constraints that many developing nations may be facing, especially budget constraints for health care and programs, the WHO recommendation of applying the test to all smear-negative cases would not be feasible in most settings.[14,18]

The impact of AFB-microscopy smear negative for TB detection has three fold: first, increased morbidity in the individual and in several cases, there may be prolonged disease episodes leading to complications such as destroyed lung syndrome and pathologic dextrocardia as reported in a rare case report;[18] second, increased incidence of the disease, for many undetected smear-negative TB, would progressively become infectious and transmit within the community leading to multidrug resistant TB or may even be resistant strain from inertia; finally, the economic impact on the household is magnified by repeated visits to health-care facilities, multiple differential diagnostic testing, treatments, and loss of earnings due to multiple cost for health care and persistent morbidity. These are theoretical and some research reasons why microscopy testing for TB may be amplifying the need for GeneXpert rollout.

The GeneXpert assay has also increased the early detection of MDR-TB, particularly when applied to high-risk groups in accordance with the WHO recommendations. Before the application of this assay, patients at high risk for MDR-TB would have to be referred to a tertiary setting and wait for undetermined periods for results of phenotypic drug susceptibility testing, resulting in high loss to follow-up and delays in treatment initiation. The line probe assays for MDR diagnosis have also largely been limited to tertiary centers in low-income countries. Without Xpert testing, this patient would have received a first-line treatment regimen for a minimum of six months before being tested for drug-resistant TB using the culture technique. This practice which is according to WHO guidelines during the first cohort period, would lead to treatment failure and complications such as destroyed lung syndrome.[19]

Clinicians are aware that a negative Xpert test does not exclude a TB diagnosis.[14]

The cost of the GeneXpert has been reported to cost 20 USD for each patient.[14] This has not been a limitation in our study because the service has been supported for free by government and international partners.

Scale-up of GeneXpert testing is still ongoing in Nigeria. In India, it was noted that GeneXpert testing would cost all smear-negative TB suspects to the tune of the entire national health-care budget.[20]

Our study is consistent with other studies which have suggested the benefit of Xpert in smear-negative patients in developing countries.[16,20-22] Most of these studies have been carried out in Africa, where there is a substantially higher HIV burden. Three studies have been reported from low-HIV prevalence regions in Asia and supported the advantage in using GeneXpert as well.[22-24]

The studied TB centre was recently upgraded in 2017 for the detection of TB drug-resistant strains using GeneXpert, with the support from an international organization in partnership with NTBLC of the Federal Ministry of Health. At this point in time, it was a pilot implementation program to improve TB detection in the state. The patients who were suspected of drug-resistant TB bacilli are referred to the Regional TB center for treatment and culture monitoring as depicted in cohort 2 [Tables 2 and 3]. Through the local centre upgrade, patients could obtain MDR-TB therapy in the centre about 50% of MDR-TB patients [Table 3], reducing travel security risk, cutting costs, and preventing the spread of MDR-TB among immediate family members and the population at risk. Before late 2016, patient samples were collected for suspected cases based on the national guideline for drug-resistant tests after meeting the clinical criteria, to be sent to the National TB center for testing using the TB culture method that was highly sensitive but riddled with delays. These efforts were costly and also rippled with delays before results would come for patients to obtain care. Delays in results from the referral system were partly due to the culture of the sample and time lag for the significant growth of the bacilli in addition to the drug sensitivity test. However, culture and drug sensitivity for bacilli is not completely relegated but is still used for monitory MDR-TB, especially for those who have complications following treatment for multidrug-resistant TB.

From local experience, GeneXpert cannot be used for very bloody sputum or samples that are too thick with tissue debris, or very proteinaceous samples. It was discovered that the content of these types of samples interferes with the amplification of the gene and proteins of the TB bacilli, and some large protein molecules and debris may block the probes of the machine. These observations serve as reasons for retaining the microscopy-AFB technique for preliminary testing [Cohort 3, Table 2] for support. These factors make maintenance and recalibration very delicate and costly. Some false-positive rifampicin Tuberculosis resistant strain has also been reported, so the CDC recommends reporting GeneXpert-Rifampin resistance as a preliminary result pending sequencing for resistance mutations.[25] Other limitation includes recurrent Cartridge and chemical diluent replacement

which is not cost-effective because their replacement must be shipped from abroad. Despite these limitations, GeneXpert has improved the detection rate in our centre and enhanced MDR-TB detection.

Conclusion

The GeneXpert detection rate of TB and multidrug-resistant (MDR) strains could be improved through global support and partnership for pilot models, such as our experience using Genexpert as first-line testing policy as well as upgrading the testing systems for TB detection in suburban and rural communities located within low-income countries. This strategy would improve public health care for TB and prevent the community transmission of MDR-TB strains resulting from using costly and time-consuming referral systems.

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Conflicts of interest

There are no conflicts of interest.

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